



Antibody–Drug Conjugate Sacituzumab Govitecan Enables a Sequential TOP1/PARP Inhibitor Therapy Strategy in Patients with Breast Cancer

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ABSTRACT

Purpose: The antibody–drug conjugate (ADC) sacituzumab govitecan (SG) comprises the topoisomerase 1 (TOP1) inhibitor (TOP1i) SN-38, coupled to a monoclonal antibody targeting trophoblast cell surface antigen 2 (TROP-2). Poly(ADP-ribose) polymerase (PARP) inhibition may synergize with TOP1i and SG, but previous studies combining systemic PARP and TOP1 inhibitors failed due to dose-limiting myelosuppression. Here, we assess the proof-of-mechanism and clinical feasibility for SG and talazoparib (TZP) employing an innovative sequential dosing schedule.

Patients and Methods: In vitro models tested pharmacodynamic endpoints, and in a phase 1b clinical trial (NCT04039230), 30 patients with metastatic triple-negative breast cancer (mTNBC) received SG and TZP in a concurrent ($N = 7$) or sequential ($N = 23$) schedule. Outcome measures included safety, tolerability, preliminary efficacy, and establishment of a recommended phase 2 dose.

Results: We hypothesized that tumor-selective delivery of TOP1i via SG would reduce nontumor toxicity and create a temporal window, enabling sequential dosing of SG and PARP inhibition. In vitro, sequential SG followed by TZP delayed TOP1 cleavage complex clearance, increased DNA damage, and promoted apoptosis. In the clinical trial, sequential SG/TZP successfully met primary objectives and demonstrated median progression-free survival (PFS) of 7.6 months without dose-limiting toxicities (DLT), while concurrent dosing yielded 2.3 months PFS and multiple DLTs including severe myelosuppression.

Conclusions: While SG dosed concurrently with TZP is not tolerated clinically due to an insufficient therapeutic window, sequential dosing of SG followed by TZP proved a viable strategy. These findings support further clinical development of the combination and suggest that ADC-based therapy may facilitate novel, mechanism-based dosing strategies.

Introduction

Use of single-agent antibody–drug conjugates (ADC) has emerged as an important approach for the treatment of refractory breast cancers including triple-negative breast cancer (TNBC), a biologically aggressive breast cancer subtype defined by the absence of estrogen and progesterone receptors and lack of human epidermal growth factor receptor 2 (*HER2*) gene amplification. Metastatic TNBC (mTNBC) is particularly challenging and is associated with substantial morbidity and short survival (1). Sacituzumab govitecan (SG), the first ADC approved for mTNBC, demonstrated higher clinical activity than standard chemotherapy in the phase III ASCENT study for patients

with previously treated mTNBC (2–5). The median progression-free survival (PFS) was 5.6 months with SG versus 1.7 months with standard chemotherapy, and the median overall survival (OS) was 12.1 months with SG versus 6.7 months with standard chemotherapy (1, 6). While use of SG monotherapy thus represents a significant advance in the treatment of metastatic breast cancer, there is a significant unmet clinical need for novel combinatorial strategies to further improve outcomes for patients with mTNBC.

Combination therapy of SG with a PARP inhibitor (PARPi) is of potential interest, given the hypothesized synergy between inhibition of TOP1 and PARP activity. This effect may result from a combination of stabilized TOP1CC, the hallmark lesion induced by TOP1 inhibitors that is typically cleared through a PARP-dependent mechanism, and inhibited repair of double-strand breaks induced by TOP1 inhibition (7–10). In xenograft models, the combination of SG and PARPi resulted in higher antitumor activity and significantly delayed time to progression compared to monotherapy ($P < 0.0017$; ref. 11). Unfortunately, multiple prior clinical trials that used combination intravenous TOP1 inhibitors (TOP1i) with PARP has resulted in dose-limiting myelosuppression that severely limited the ability to dose escalate both PARPi and traditional TOP1i, precluding this combination from moving beyond early phase studies (12, 13). However, high accumulation of SN-38 in tumor cells achieved with SG (>100 fold more SN-38 than can be achieved with intravenous TOP1i in preclinical models) together with relative sparing of normal cells suggested the possibility that delayed clearance of SN-38 in tumor

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Translational Relevance

Antibody–drug conjugates (ADC) are emerging as promising cancer therapeutics, but their unique drug delivery mechanism has not been extensively leveraged for combination therapy. While a strong mechanistic rationale supports combining TOP1 inhibitor with PARP inhibitor, systemic delivery of this combination has proved clinically challenging. We proposed that the ADC-based delivery mechanism could enhance the therapeutic window for the combination, while also creating a temporal window to enable a novel sequential dosing strategy. Our pre-clinical data support this mechanistic rationale, demonstrating strongly enhanced pharmacodynamic endpoints despite temporal separation of sacituzumab govitecan and talazoparib dosing. In the clinical trial, sequential dosing overcame dose-limiting toxicities observed with concurrent dosing, allowing successful determination of the recommended phase 2 dose. These results support further clinical development of this combination, and more broadly suggest that unique features of ADCs may facilitate novel, mechanism-based dosing strategies that render viable previously abandoned therapeutic combinations in oncology.

cells could create a temporal window for sequential use of the combination (12, 14).

We hypothesized that the tumor-selective delivery of TOP1i via the ADC mechanism would reduce nontumor toxicity, while also creating a temporal window to enable sequential dosing of SG and PARPi that further enhances the therapeutic window (Supplementary Fig. S1). We investigated this hypothesis in preclinical models and a phase 1b clinical trial in patients with mTNBC.

Patients and Methods

Cell lines and cell culture

All cell lines were obtained from the MGH Center for Molecular Therapeutics cell bank and underwent high-density SNP typing to confirm their identity. All experiments shown were performed within <6 months' passage of all lines since acquisition. Cells were maintained at 37°C in 5% CO₂. MDA-MB-468 cells (RRID:CVCL_0419) were grown in RPMI (Lonza) supplemented with 10% FBS (SAFC), 1% penicillin (Gibco), streptomycin (Gibco). WI-38 cells (RRID:CVCL_0579) were grown in DMEM (Lonza) supplemented with 10% FBS (SAFC), 1% penicillin (Gibco), streptomycin (Gibco).

Modification of the rapid approach to DNA adduct recovery assay for detection of TOP1CC

Detection of TOP1CC was performed according to the published protocol (PMID: 34408146) with minor modification. After SG and/or talazoparib (TZP) treatment, 1×10^6 cells per condition were washed with $1 \times$ PBS and lysed with 600 μ L DNazol (Invitrogen) at 4°C for 10 minutes, followed by precipitation with 300 μ L 100% ethanol. The nucleic acids were collected by centrifugation at 15,000 rpm for 10 minutes at 4°C, washed with 75% ethanol twice, and resuspended in 200 μ L TE buffer. The samples were then heated at 65°C for 15 minutes, followed by shearing with sonication (40% output for 10 seconds pulse and 10 seconds rest for four times). The samples were centrifuged at 15,000 rpm for 5 minutes at 4°C and the

supernatant was collected and treated with 100 μ g/mL RNase A (Thermo Fisher Scientific) for 1 hour at 4°C, followed by the addition of 0.1 volume of 3 mol/L sodium acetate (pH 5.5) and 2.5 volume of 100% ethanol. After 20 minutes of centrifugation at 15,000 rpm at 4°C, the DNA pellet was resuspended in 100 μ L TE buffer. The concentration of the sample was quantified by NanoDrop. Ten micrograms of DNA from each condition was digested with 50 U of micrococcal nuclease (Thermo Fisher Scientific) in the presence of 5 mmol/L CaCl₂ at 37°C for 30 minutes. The samples were then mixed with 5 $\times$ SDS sample buffer and subjected to Western blotting by using anti-TOP1 antibody (Abcam, #ab109374). Two micrograms of dsDNA for each condition was subjected to dot-blot by using Nylone membrane (Santa Cruz) for immunoblotting with anti-dsDNA antibody (Abcam, #ab27156) as a loading control to verify that amounts of DNA were digested with micrococcal nuclease.

Flow cytometry

For flow cytometry detection of γ H2AX, 1×10^6 cells were collected and fixed with 4% paraformaldehyde at 4°C for 20 minutes and then permeabilized with 0.25% Triton X-100 in PBS. After blocking with 2% BSA in PBS at 4°C for 20 minutes, the cells were stained with Alexa Fluor 488 conjugated with anti-phospho Histone H2A.X antibody (EMD Millipore, clone JBW301, #05-636-AF488) at 4°C for 1 hour. The cells were then washed twice with 2% BSA in PBS and counterstained with DAPI (Abcam, #ab228549). For flow cytometry detection of apoptotic cells, 1×10^6 cells were collected, and the staining was performed by using eBioscience™ Annexin V Apoptosis Detection Kit APC (Invitrogen, #88-8007) according to the manufacturer's instruction. Samples were examined using a FACSAria flow cytometer. Analysis was conducted with FlowJo (RRID:SCR_008520) using Cell Quest software (Becton Dickinson, Franklin Lakes, NJ).

Immunofluorescence

To visualize TOP1CC by immunofluorescence, cells were plated into 96-well plates having #1.5 glass coverslips (Cellvis, P98-1.5H-N). Cells were then treated with SG and/or TZP, washed with 1X PBS, and fixed with 4% paraformaldehyde (PFA) at 37°C for 10 minutes. Thereafter, cells were washed three times with 1X PBS and permeabilized with 0.1% NaCitrate/0.1% Triton X-100 in ddH₂O at room temperature for 5 minutes. Cells were then treated with 0.5% SDS in PBS at room temperature for 5 minutes and washed five times with 0.25% BSA/0.1% Tween-20 in PBS. After blocking in 2% BSA/10% normal goat serum/0.1% Tween-20 in PBS at room temperature for 30 minutes, cells were incubated with anti-TOP1CC antibody (EMD Millipore, MABE1084) diluted 1:100 in blocking buffer at room temperature for 1 hour. Following incubation with an Alexa Fluor 488 secondary antibody (Jackson ImmunoResearch) at room temperature for 30 minutes and DAPI counterstaining, cells were imaged using a Nikon A1R confocal microscope with a $\times 60$ oil immersion objective. Images were compiled and quantified using ImageJ software (RRID: SCR_003070).

Clinical study design

In a phase 1b/2, open-label study, SG was administered in combination with TZP for patients with mTNBC (clinicaltrials.gov #NCT04039230). All details of the trial and consent were reviewed approved by the appropriate Institutional Review Board (Dana-Farber Harvard Cancer Center IRB). The trial was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization guidelines for Good Clinical Practice and

local regulations. Patients or legal guardians provided written informed consent, and assent was obtained per institutional guidelines. Participants were not compensated.

The standard 3 + 3 design was used in this study. Starting with three patients at the lowest dose level, three patients were enrolled, treated, and monitored during the dose-limiting toxicity (DLT) evaluation period. Several increasing dose levels are then examined sequentially. If one patient experienced a DLT, then up to three more patients are tested at the same dose level. Only one out of the six of these patients experienced a DLT for the testing to move on to the next higher dose level. At any dose level, if two patients tested experienced a DLT, that dose level was deemed to have unacceptable toxicity and abandoned from further study. In this event, testing of the dose level immediately below the poorly tolerated dose level was expanded, if necessary, to include a total of six patients. The process of escalation and de-escalation was repeated until the maximum tolerated dose (MTD) was reached. Patients with inadequate data for DLT decision and/or tumor assessment could be replaced to have enough number of patients for decision making.

DLT was defined based on the first treatment cycle as any of the following events, possibly related to one or both study drugs: any death not clearly due to the underlying disease or extraneous causes, Hy's law, neutropenic fever, any grade 3+ nonhematologic toxicity, any grade 4+ neutropenia or thrombocytopenia >7 days, grade 3+ thrombocytopenia with bleeding grade 3+ nausea/vomiting or diarrhea >72 hours with adequate antiemetic and other supportive care, grade 3+ fatigue ≥1 week, and grade 3+ electrolyte abnormality that lasts >72 hours. All adverse events (AEs) of the specified grades were considered DLTs except those that are clearly and incontrovertibly due to disease progression or extraneous causes. For patients with hepatic metastases, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 8× institutional upper limit of normal (ULN) or AST or ALT > 5× institutional ULN for ≥14 days was considered as DLT. The DLT evaluation period was during the first treatment cycle. Patients must have received two thirds (≥66%) or higher of scheduled TZP doses during the first cycle to be considered evaluable for a DLT. Those patients experiencing DLT underwent dose reduction, but all eventually remained on study until disease progression.

Treatment cycles were continued until unacceptable toxicity or progression of disease at the discretion of the treating physician. Within 4 weeks prior to the first dose of study treatment administration, baseline evaluations were completed, which included patient medical and surgical history, a physical examination with vital signs and performance evaluation, laboratories, and tumor assessment imaging. Once on active treatment, patients were administered SG and TZP over a 21-day cycle. The cycles were continued in the absence of unacceptable toxicity or progression of disease. During treatment, study procedures include physical examinations, vital signs, blood labs, serum samples, concomitant medications, adverse events, EKG, and restaging scans. All patients were monitored closely over the course of their treatment and NCI CTC v5.0 was used to grade all adverse events and provide dose reduction, delay, or cessation guidelines in the event of treatment-related toxicity. The standard 3 + 3 dose-escalation design was used in this study, with additional patients allowed to enroll for research purposes at the discretion of the principal investigator.

Study population

Patients enrolled were 18 years of age or older with histological or cytological confirmation of TNBC as determined by the local institution following ASCO-CAP guidelines; however, patients with "low" estrogen receptor/progesterone receptor (ER/PR) staining (up

to 10% low-level staining) were eligible after approval of the study principal investigator (PI). Metastatic disease was documented by CT or MRI imaging, and measurable disease by CT/MRI was required. All patients had an Eastern Cooperative Oncology Group (ECOG) performance score of ≤1 at screening, and adequate bone marrow, hepatic, and renal function. Patients were at least 2 weeks beyond prior anticancer treatment. Patients who received prior irinotecan or ADC backbone with SN-38 or other TOP1i were excluded. One patient received prior ladiratuzumab vedodin.

Study medications

SG was administered intravenously on days 1 and 8 of each 21-day cycle on an outpatient basis in this cohort. TZP was provided as capsules for oral administration. When on active treatment, other anticancer treatments were not permitted during this study. Palliative and/or supportive medications and procedures were permitted at the physician's discretion, including G-CSF support.

Endpoints

Safety and tolerability of SG in combination with TZP was evaluated from adverse events, standard safety laboratories, physical examination, vital signs, and EKG. All adverse events and abnormal laboratories were classified for severity using NCI CTCAE v5.0 toxicity grades. Treatment efficacy was evaluated from CT or MRI scans every 9 weeks using Response Evaluation Criteria in Solid Tumors (RECIST V1.1) criteria to classify tumor response, time to onset of objective response, duration of objective response, PFS, and OS and the results were documented. After the discontinuation of study treatment, all patients were followed every 8 weeks for survival follow-up. Follow-up visits could be in-clinic or by telephone and were meant to document any further therapy administered for the patient's breast cancer. Adverse event reporting continued for 30 days after the last dose of study treatment.

Biomarker assessment

IHC assessment of biomarkers (TILs, Ki67, TROP2) was done by a pathologist, blinded to the study results, per standard methodology. For TROP2 staining, AF650 antibody (R&D Systems, Minneapolis, MN), overnight incubation at 4°C, 1:100 dilution, was utilized. Tumor cell membrane TROP2 expression was categorized based on a histochemical score (H-score), a numerical value represented by a weighted summation of percent staining that accounts for both the staining intensity and the percentage of cells at that intensity, as defined by Bardia and colleagues (15). TROP2 cutoffs were as follows: <100 (low); 100 to 200 (medium); and 201 to 300 (high). High TILs was defined as >20% of cells, and Ki67 high as ≥30% expression. Plasma-based genotyping was performed with analytically validated Guardant360 assay.

Data availability

Deidentified patient data reported in the manuscript may be requested from the corresponding authors and will be available for up to 6 years after publication. Release of deidentified data involves a formal review process that includes ensuring that any transfer is in compliance with the IRB. The requesting investigator will be required to sign a data release form prior to transfer.

Results

We first used cell-based TNBC models to test the hypothesis that sequential dosing of SG and PARPi with TZP would be sufficient to

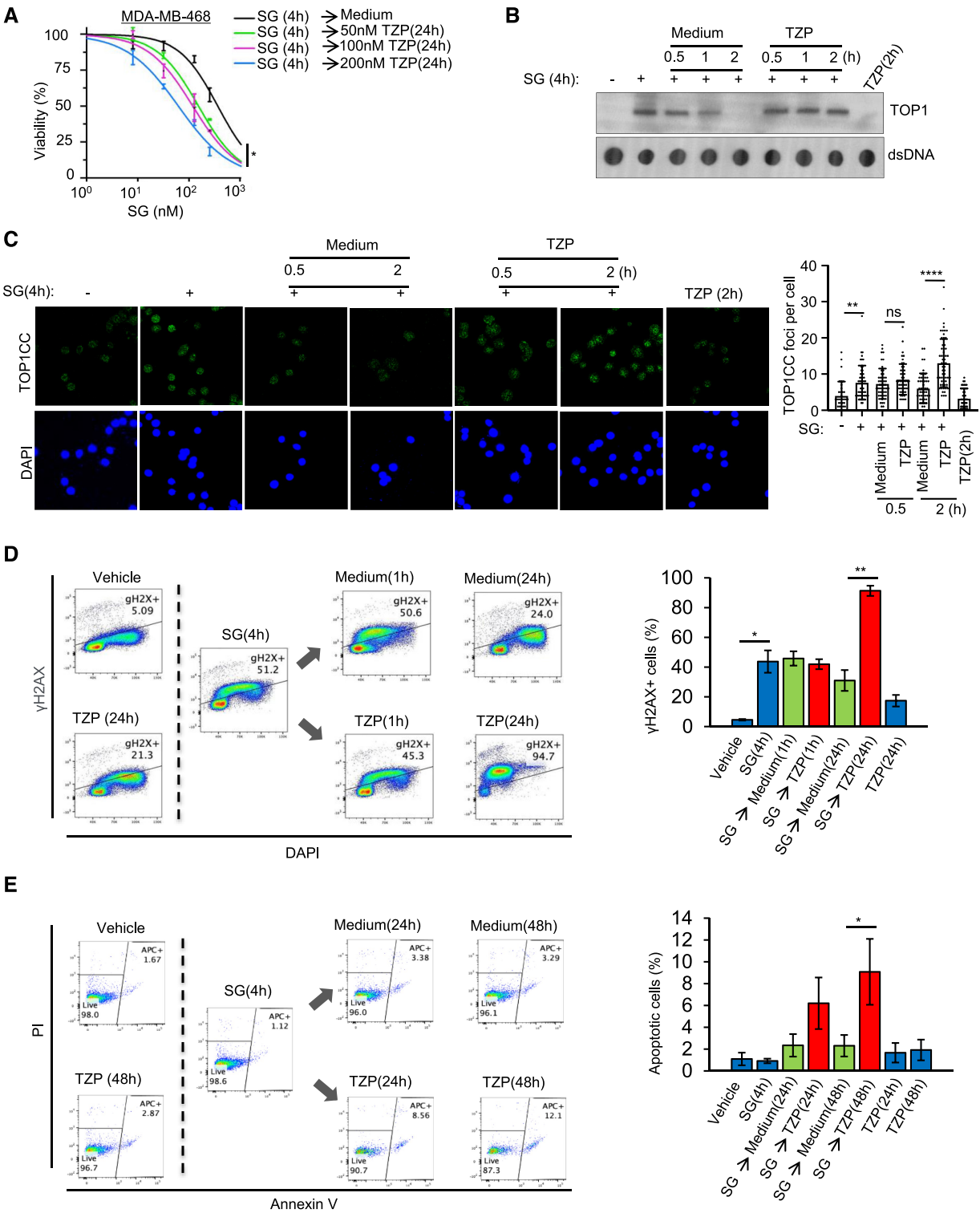


Figure 1. Sequential dosing of SG and PARPi stabilizes TOP1CC and induces DNA damage and cell death. **A**, Dose-response curves of human MDA-MB-468 TNBC cells treated with different concentrations of SG for 4 hours followed by washout, then treated with either vehicle or indicated doses of TZP for 24 hours followed by medium change and assessment of viability at 72 hours. Values plotted represent the mean of quadruplicate wells and an average of three independent experiments. Error bars indicate SD. *P* value calculated by two-way ANOVA. *, *P* < 0.05. **B**, Sequential SG then TZP results in persistent TOP1 covalently bound to DNA, as assessed by the RADAR assay (see Patients and Methods). MDA-MB-468 cells were treated with SG (50 nmol/L) (Continued on the following page.)

allow PARPi-induced stabilization of TOP1CC and enhance DNA damage and cell killing. We exposed TROP2-expressing TNBC cells or normal diploid cells to SG, followed by washout, then PARPi treatment with TZP. Despite the temporal separation, SG followed by PARPi (at a minimally toxic dose) showed a dose-dependent enhancement of SG toxicity at clinically achievable doses (Fig. 1A; Supplementary Fig. S2A). In contrast, normal diploid cells showed little SG sensitivity and no appreciable enhancement with the addition of PARPi (Supplementary Fig. S2B). We next quantified TOP1CC by two methods: the rapid approach to DNA adduct recovery (RADAR) assay, which detects TOP1 covalently bound to DNA, and immunofluorescence. We found that TOP1CC as assessed by either method were detectable immediately following SG washout but resolved entirely within 2 hours, whereas in the presence of sequential PARPi, no resolution of TOP1CC was observed (Fig. 1B and C). Sequential PARPi enhanced dose-dependent SG toxicity and stabilized TOP1CC in multiple TNBC models (Supplementary Fig. S2C and S2D). Persistent TOP1CC are expected to lead to DNA damage including double-strand DNA breaks, which we quantified using flow cytometry to assess phosphorylated histone H2AX (γ H2AX) staining. In the absence of PARPi, washout of SG led to resolution of DNA damage within 24 hours, whereas with sequential addition of PARPi following SG washout, cells continued to accumulate high levels of DNA damage (Fig. 1D). Correspondingly, apoptosis was consistently and significantly increased by the addition of PARPi post SG washout (Fig. 1E). Collectively, these findings support the rationale for sequential SG/PARPi as a means to enhance tumor cell killing, with the potential to limit off-target toxicity and allow improved safety.

We then designed and conducted an investigator-initiated phase 1b multicenter study to evaluate the safety/tolerability and efficacy of SG and the PARP inhibitor TZP combination therapy for patients with metastatic TNBC (clinicaltrials.gov #NCT04039230). The primary objective was to assess the DLT rate and MTD of SG in combination with TZP in patients with mTNBC and to select the recommended phase 2 dose (RP2D) of this combination. Between October 2019 and April 2021, 30 patients were enrolled in this dose-escalation phase 1b clinical trial. Initially, seven patients were enrolled and received concurrent SG and TZP, but subsequent enrollment in this concurrent cohort was discontinued due to significant DLT. Consequently, an additional 23 patients were enrolled and received sequential SG followed by TZP (see CONSORT diagram, Supplementary Fig. S3). There were no significant differences in baseline characteristics between the sequential and concurrent cohorts, including median age, race, ECOG PS, prior lines of therapy, and site of metastasis (Supplementary Table S1).

The concurrent dosing schedule was associated with significant toxicity, with five of seven patients (71.4%) experiencing DLTs, including febrile neutropenia. In contrast, no patient experienced a DLT with the sequential schedule (Fig. 2A). Similarly, the incidence of adverse effects, including neutropenia, anemia, thrombocytopenia,

and diarrhea, was higher in the concurrent cohort compared with the sequential cohort (Fig. 2A; Supplementary Table S2). Sequential dosing was deemed feasible and the study met its prespecified primary endpoint, with the RP2D being SG 10 mg/kg days 1, 8 and TZP 1 mg days 15 to 21, every 21 days. The DLT criteria, frequency and associated dose levels are summarized in Patients and Methods and in Supplementary Tables S3 and S4.

In the efficacy analysis, two of seven patients in the concurrent schedule cohort experienced a partial response, while 8 of 19 evaluable patients in the sequential cohort had a confirmed partial response (Fig. 2B; Supplementary Table S5). Furthermore, 15 of 19 evaluable patients in the sequential cohort experienced clinical benefit compared to three of seven in the concurrent cohort, and the median PFS was higher in patients enrolled in the sequential versus concurrent cohort: 7.6 months versus 2.3 months (Fig. 2C; Supplementary Table S5). The overall survival was also longer in patients enrolled in the sequential cohort (11.1 months) as compared to those in the concurrent cohort (4.3 months), as was time-on-treatment (Fig. 2D; Supplementary Table S5).

Analysis of tumor histology and immunohistochemistry was consistent with an on-target effect of SG, demonstrating that TROP2 tumor cell membrane expression (H-score; see Patients and Methods) was medium or high in >80% of patients experiencing objective response, while nearly 50% of those experiencing progressive disease as best response had low TROP2-expressing tumors (Fig. 2E; Supplementary Fig. S4A). TILs and Ki67 were not associated with response in this small metastatic cohort, in contrast to the findings of the NeoSTAR trial of SG monotherapy for early-stage TNBC (Supplementary Fig. S4B; ref. 16). Genomic analysis of tumors from treated patients reflected the anticipated somatic alterations in TNBC (Fig. 2B). Additionally, proportionally higher objective response rates were observed in tumors with known germline or somatic *BRCA1/2* mutation (Fig. 2B; Supplementary Table S6), though caution should be exerted in overinterpretation given small numbers. Thus, concurrent dosing of SG and TZP was deemed not clinically feasible, while sequential dosing of this combination successfully met the prespecified primary endpoints.

Discussion

Here, we demonstrate the mechanistic rationale and clinical proof-of-principle supporting sequential dosing of SG and PARPi to mitigate toxicity and enhance efficacy. The potential synergy between PARPi and TOP1i has been long recognized (17). PARP may contribute in multiple ways to limiting TOP1i-induced DNA damage, including by activating proteasomal degradation of TOP1CC and by promoting microhomology-mediated end joining and homologous recombination-mediated repair of DNA double-strand breaks that

(Continued.) for 4 hours followed by washout, then either cultured with or without TZP (100 nmol/L) for the indicated times and processed for RADAR. Detection of dsDNA by dot-blot serves as a loading control. **C**, Confocal immunofluorescence with TOP1CC-specific antibody. MDA-MB-468 cells were treated with 50 nmol/L of SG for 4 hours followed by washout, then cultured with or without 100 nmol/L of TZP for the indicated times. At right, quantification of TOP1CC foci per cell. At least 50 cells were counted for each condition. Error bars indicate SD. *P* value calculated by two-way ANOVA. **, *P* < 0.01; ****, *P* < 0.0001; ns, no significance. **D**, Accumulation of DNA damage induced by sequential dosing of SG and TZP. MDA-MB-468 cells were treated as indicated, fixed and stained with anti- γ -H2AX antibody and DAPI, then analyzed by flow cytometry. Left: representative flow cytometry plots. Right: quantification of percentage of γ -H2AX positive cells from duplicate samples in two independent experiments. Error bars indicate SD. *P* value calculated by Student *t* test. *, *P* < 0.05; **, *P* < 0.01. **E**, Increased apoptosis induced by sequential dosing of SG and TZP. MDA-MB-468 cells were stained with propidium iodide and Annexin V following the indicated treatment. Left: representative flow cytometry plot. Right: quantification of percentage of apoptotic cells from duplicate samples in two independent experiments. Error bars indicate SD. *P* value calculated by Student *t* test. *, *P* < 0.05.

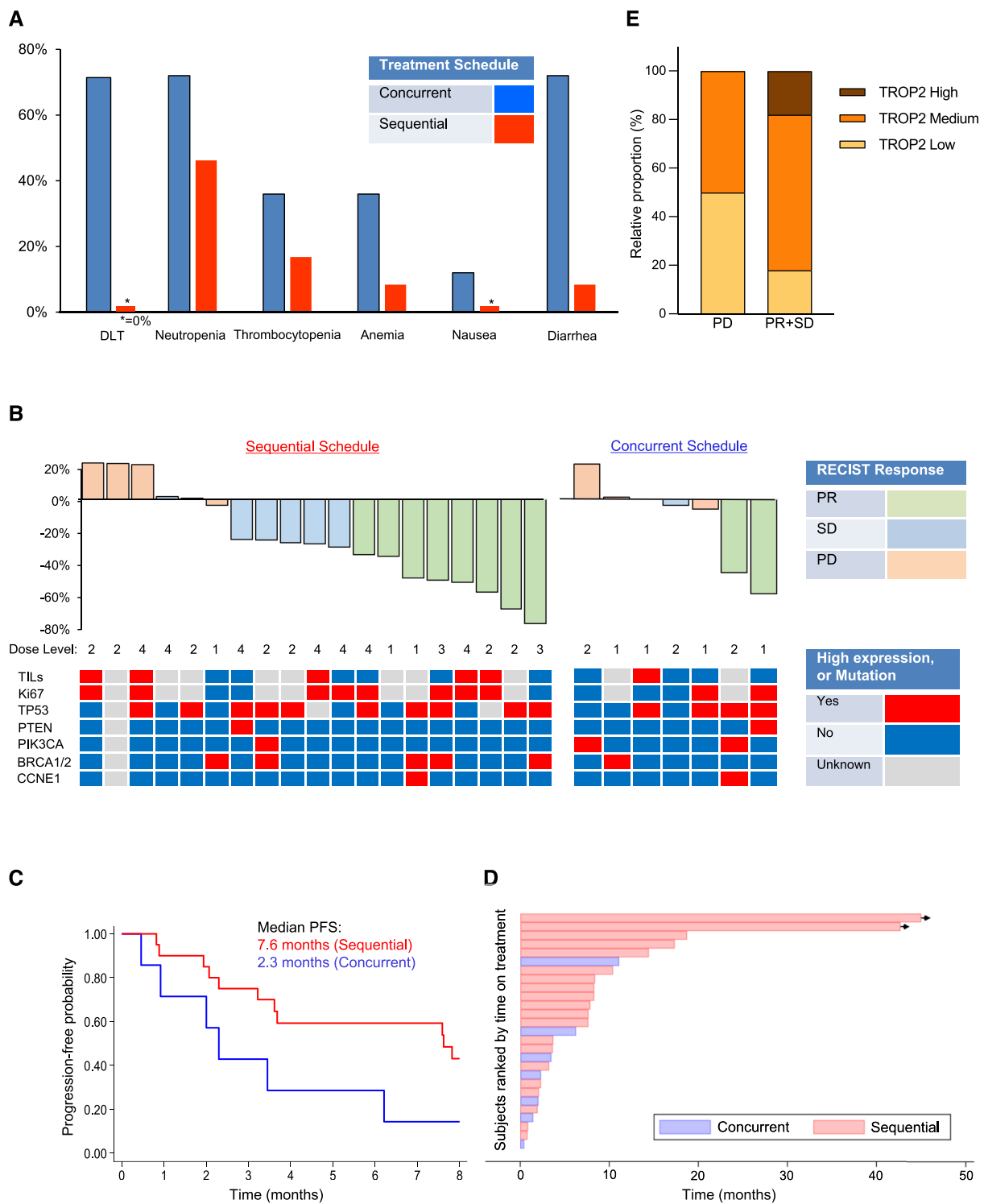


Figure 2. Sequential dosing of SG and PARPi is associated with lower incidence of adverse effects and higher clinical efficacy than concurrent dosing. **A**, Percentage of patients experiencing DLTs and significant grade 3/4 adverse events with concurrent (blue) and sequential (red) schedule. **B**, Waterfall plot indicating the best percent change from baseline in the sum of the diameters of the target lesions (longest diameter for non-nodal lesions and short axis for nodal lesions), per RECIST V1.1, with sequential (left) and concurrent (right) schedule. Below are shown tumor biomarker assessments including TROP2 staining by IHC; TILs, Ki67 staining by IHC; and mutation of *TP53*, *PTEN*, *PIK3CA*, *BRCA1/2*, and *CCNE1* assessed by validated plasma-based genotyping assay (Guardant³⁶⁰). **C**, Kaplan-Meier analysis of PFS with sequential (red line) versus concurrent (blue line) schedules. **D**, Swimmers' plot illustrating (Continued on the following page.)

result from persistent TOPICC (12). Consistent with these models, we find that TOPICC induced by SG are resolved to baseline within 2 hours of SG washout in vitro, but they persist in the presence of PARPi, leading to progressive accumulation of DNA damage and ultimately apoptosis.

Unfortunately, despite a strong mechanistic rationale for the combination of these two active therapeutic classes, systemic delivery of PARPi and TOP1i is associated with an insufficient therapeutic window and proved unsuccessful in multiple clinical trials due to severe toxicity (13). Consistent with our findings involving concurrent dosing of SG and TZP, the combination of SG with rucaparib in a phase 1 clinical trial (SEASTAR) was associated with unacceptable toxicity. Despite utilizing a lower dose of SG than that we employed in our study (6 mg/kg), significant myelosuppression was observed with concurrent PARPi dosing, thus halting the study and further clinical development of the combination (18). Consistent with these data, our clinical trial demonstrated that concurrent administration of SG and TZP produced unacceptable myelosuppression. Taken together, these observations suggest that exploiting the relative tumor-specificity of payload TOP1i delivery by the ADC was not sufficient alone to render the combination tolerable. However, we hypothesized that this specificity and high intratumoral SN-38 delivery via ADC (14) would also provide a temporal window, enabling a novel dosing strategy with sequential treatment that we found indeed demonstrated improved tolerability and allowed successful completion of this dose-escalation study. Accordingly, despite the combination therapy, the incidence of adverse events, including myelosuppression and diarrhea with the SG + PARPi sequential schedule, was generally comparable to those observed with SG monotherapy in prior pivotal clinical trials (1, 19).

The results of this study have broad implications for combination therapy with ADCs. Sequential delivery of a DNA damaging agent and a repair inhibitor could also be utilized for other ADCs, particularly those with TOP1i payloads such as trastuzumab deruxtecan, datopotamab deruxtecan, and patritumab deruxtecan, all of which are either approved or in advanced stages of clinical development in oncology (20). More broadly, the tumor-selective delivery of antibody-targeted drugs may facilitate combinations and successful mechanism-based dosing strategies with diverse other agents, including the possibility of revisiting previously abandoned options in combination with novel ADCs.

Naturally, this single-arm dose-escalation study cannot address the question as to whether combination therapy has higher efficacy than previously reported for single agent SG, particularly given potential differences in study population, prior lines of therapy, and other confounding factors (Supplementary Table S7). Furthermore, display of response and PFS data for concurrent versus sequential schedules is not meant as a strict comparison of efficacy for the two schedules, but rather demonstrates that experiencing a DLT (and thus having treatment interruption and dose reduction) may impact treatment benefit. Nonetheless, the results of our study provide critical proof-of-principle and support further clinical development of this therapeutic combination, including a phase 2 dose expansion study and a future randomized phase 2 clinical trial.

In summary, sequential dosing of SG and PARPi, leveraging the selective drug delivery mechanism of SG to minimize toxicity while

maintaining efficacy, demonstrated encouraging evidence of clinical activity with objective responses among pretreated patients with mTNBC. The dose-escalation study successfully completed enrollment with R2PD and schedule. Collectively, these preclinical and clinical results highlight how mechanistic insights and innovative scheduling can be utilized to develop promising drug combinations, including previously rejected combinations, for patients with solid tumors.

Authors' Disclosures

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Authors' Contributions

A. Bardia: Conceptualization, resources, funding acquisition, investigation, writing—original draft, writing—review and editing. S. Sun: Data curation, formal analysis, investigation, methodology. N. Thimmiah: Data curation, formal analysis, investigation, methodology. J.T. Coates: Data curation, formal analysis, investigation, methodology. B. Wu: Data curation, formal analysis, supervision, investigation, methodology. R.O. Abelman: Data curation, project administration. L. Spring: Investigation. B. Moy: Investigation. P. Ryan: Data curation. M.N. Melkonyan: Data curation, investigation. A. Partridge: Investigation. D. Juric: Investigation. J. Peppercorn: Investigation. H. Parsons: Investigation. S.A. Wander: Investigation. V. Attaya: Investigation. B. Lormil: Investigation. M. Shellock: Investigation. A. Nagayama: Data curation, formal analysis. V. Bossuyt: Formal analysis, investigation. S.J. Isakoff: Investigation.

(Continued.) time-on-treatment for patients enrolled in concurrent and sequential cohorts. Two patients remain on-treatment as of the data cutoff date. E, Distribution of TROP2 tumor cell membrane staining as quantitated by H-score (see Patients and Methods) in patients experiencing PD ($n = 6$) versus PR/CR ($n = 17$). H-score cutoffs: low (<100); medium (100–200); high (201–300). PD, progressive disease; PR, partial response; SD, stable disease.

S.M. Tolaney: Investigation. **L.W. Ellisen:** Conceptualization, resources, supervision, funding acquisition, investigation, writing—original draft, writing—review and editing.

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Note

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